Osmoregulation, vasopressin, and cAMP signaling in autosomal dominant polycystic kidney disease

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Abstract: PURPOSE OF REVIEW: Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent inherited nephropathy. This review will focus on the vasopressin and 3'-5'-cyclic adenosine monophosphate (cAMP) signaling pathways in ADPKD and will discuss how these insights offer new possibilities for the follow-up and treatment of the disease. RECENT FINDINGS: Defective osmoregulation is an early manifestation of ADPKD and originates from both peripheral (renal effect of vasopressin) and central (release of vasopressin) components. Copeptin, which is released from the vasopressin precursor, may identify ADPKD patients at risk for rapid disease progression. Increased levels of cAMP in tubular cells, reflecting modifications in intracellular calcium homeostasis and abnormal stimulation of the vasopressin V2 receptor (V2R), play a central role in cystogenesis. Blocking the V2R lowers cAMP in cystic tissues, slows renal cystic progression and improves renal function in preclinical models. A phase III clinical trial investigating the effect of the V2R antagonist tolvaptan in ADPKD patients has shown that this treatment blunts kidney growth, reduces associated symptoms and slows kidney function decline when given over 3 years. SUMMARY: These advances open perspectives for the understanding of cystogenesis in ADPKD, the mechanisms of osmoregulation, the role of polycystins in the brain, and the pleiotropic action of vasopressin.

DOI: https://doi.org/10.1097/MNH.0b013e3283621510

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Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent inherited nephropathy. This review will focus on the vasopressin and 3′-5′-cyclic adenosine monophosphate (cAMP) signaling pathways in ADPKD and will discuss how these insights offer new possibilities for the follow-up and treatment of the disease.

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Summary
These advances open perspectives for the understanding of cystogenesis in ADPKD, the mechanisms of osmoregulation, the role of polycystins in the brain, and the pleiotropic action of vasopressin.

Keywords
collecting duct, osmoregulation, polycystins, V2 receptor antagonist, vasopressin

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent inherited nephropathy (prevalence rv1 : 1000), characterized by the development of multiple cysts in the kidneys. Mutations in PKD1 and PKD2 account for 85 and 15% of the affected families, respectively. The PKD1 and PKD2 genes encode integral membrane proteins, polycystin-1 and polycystin-2, which form a complex localized in various cellular domains including the primary cilium wherein the polycystins mediate calcium fluxes in response to mechanical or chemical stimuli. Mutations in PKD1/PKD2 alter intracellular calcium homeostasis and lead to cystogenesis by increased cell proliferation, abnormal fluid secretion, and dedifferentiation [1–3].

The ADPKD cysts derive from 1 to 3% of the nephrons. The cysts may involve all nephron segments, but cysts of collecting duct origin predominate [4,5]. Many cysts likely develop in utero, but may only become clinically detectable years later. The prospective follow-up of ADPKD patients with MRI examinations has established that, in adults, cysts increase at 'an average' or 'an average and stable' rate of approximately 5% per year [6]. Total kidney volume (TKV) and cyst volume progression are the strongest predictors of renal function decline in ADPKD [7]. More than 50% of patients with ADPKD present a slow progression to end-stage renal failure that occurs usually in the sixth or seventh decade. Apart from symptomatic

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KEY POINTS

- Defective osmoregulation is an early manifestation of ADPKD and originates from both peripheral (resistance to vasopressin) and central (impaired release of vasopressin) components.
- Copeptin, which is released from the vasopressin precursor, may identify ADPKD patients at risk for rapid disease progression.
- Increased levels of cAMP in tubular cells, reflecting modifications in intracellular calcium homeostasis and abnormal stimulation of the vasopressin V2R, play a central role in cystogenesis.
- Blocking the V2R lowers cAMP in cystic tissues, slows renal cystic progression and improves renal function in preclinical models.
- The TEMPO 3:4 phase III clinical trial has shown that the V2R antagonist tolvaptan blunts kidney growth, reduces associated symptoms and slows kidney function decline when given over 3 years in ADPKD patients.

measures, there is no effective treatment able to slow disease progression. ADPKD is responsible for 4–10% of the patients requiring a renal replacement therapy.

The development of cysts in ADPKD requires tubular cell proliferation, abnormalities in the extracellular matrix and transepithelial fluid secretion (Fig. 1). Increased concentrations of 3^{0}-5^{0}-cyclic adenosine monophosphate (cAMP) play a major role in renal cystic disease progression [2]. Stimulation of the vasopressin V2 receptor (V2R) by the antidiuretic hormone arginine vasopressin (AVP) is the major regulator of adenylyl cyclase activity and source of cAMP production in the distal nephron. Haploinsufficiency in polycystin-1 has been associated with excessive vasopressin signaling and inappropriate antidiuresis in mouse [8]. Increased levels of cAMP and cAMP-target genes have been observed in the cystic kidneys of various rodent models. The increased cAMP levels may arise from decreased intracellular Ca^{2+} concentration caused by mutations in polycystins, via the downregulation of calcium-dependent phosphodiesterase PDE1 and stimulation of the Ca^{2+}-inhibitable adenylyl cyclase 6 (AC6) [2]. In turn, increased cAMP stimulates the proliferation and growth of ADPKD cells and drives chloride and fluid secretion (Fig. 1).

The importance of the V2R–cAMP pathway in mediating renal cystic disease has been demonstrated in animal models of PKD. These studies motivated a phase III clinical trial investigating the effect of the selective V2R antagonist tolvaptan (OPC-41061) in ADPKD patients [9,10**]. In this review, we will focus on vasopressin and cAMP signaling pathways in ADPKD and will discuss how these insights offer new possibilities for follow-up and treatment of the disease.

OSMOREGULATION IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

That ADPKD is associated with defective osmoregulation has been known for decades. Defective urinary concentration is frequently observed in ADPKD patients and is more severe in patients harboring large kidneys on ultrasound analysis [11]. A peripheral resistance to vasopressin has been suggested, potentially explained by cystic lesions affecting the interstitial osmotic gradient driving water reabsorption [12*].

Recently, Ho et al. [13*] investigated the osmoregulation parameters in adult and pediatric ADPKD patients with intact glomerular filtration rate (GFR). In comparison with nonaffected controls, ADPKD patients showed a significant defect both in the release of vasopressin in response to plasma osmolality (central component) and in the V2R-mediated response (nephrogenic component). The peripheral resistance to vasopressin is correlated with TKV as assessed by MRI in adults. However, the presence of cysts or their number is not a prerequisite for the osmoregulation defect in ADPKD children [13*]. In fact, developmental studies in diphenylthiazole-induced rats [14] and cpk mice [15] have shown that the urinary concentrating defect precedes renal cyst development. Defective cellular processes have been evoked [15], supported by evidence for altered vasopressin downstream signaling in heterozygous Pkd1 mice [8]. Although baseline plasma vasopressin levels were similar in ADPKD patients and controls, the relationship between plasma osmolality and vasopressin, obtained after water deprivation, was severely blunted in ADPKD patients. This observation suggests that ADPKD patients have a central defect altering the release of AVP in response to increased osmolality [13*].

The fact that both Pkd1/Pkd2 (mouse) and PKD1/PKD2 (human) are expressed in the supraoptic, suprachiasmatic, and paraventricular nuclei that synthesize and release vasopressin could provide a basis for a central osmoregulation defect in ADPKD [13*]. The osmosensitivity of vasopressin neurons is conferred by mechanosensitive cation channels that include TRPV4 [16]. There is evidence that polycystin-2 interacts with TRPV4 to form a mechanosensor driving calcium transients in vitro [17]. One could hypothesize that a defect in the complex

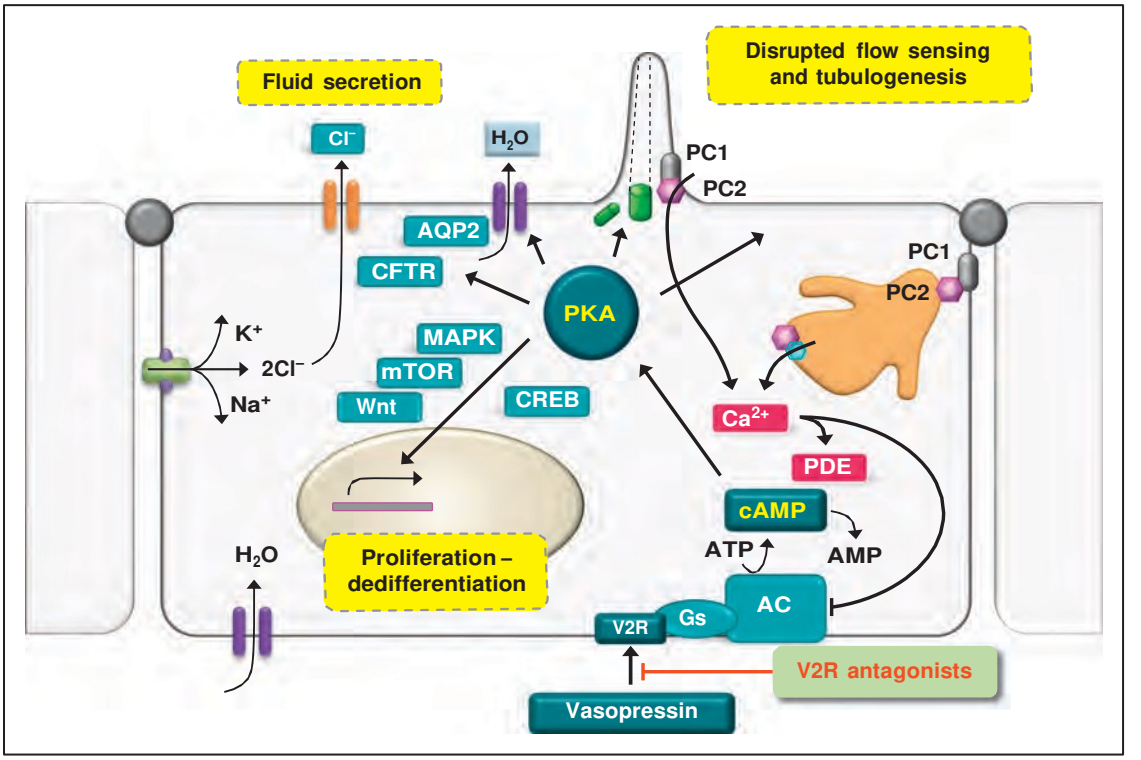

FIGURE 1. Role of 3′-5′-cyclic adenosine monophosphate (cAMP) in autosomal dominant polycystic kidney disease cyst-lining epithelial cells. A cyst-lining tubular cell (from the collecting duct) is depicted, with tight junctions delineating the apical and basolateral poles. The complex involving polycystin-1 (PC1) and polycystin-2 (PC2) mediates calcium fluxes in response to stimuli sensed by the primary cilium (apical pole). Disruption of the PC1–PC2 complex is involved in the alteration of intracellular Ca^{2+} levels. The ADPKD cyst-lining cells show an increased concentration of cAMP, probably reflecting reduced intracellular calcium levels [which stimulates Ca^{2+}-inhibitable adenylyl cyclase (AC) and/or inhibits the Ca^{2+}-dependent phosphodiesterase (PDE)] and stimulation of the vasopressin V2 receptor (V2R) pathway. The increased cAMP levels stimulate protein kinase A (PKA)-mediated phosphorylation of various mediators, leading to disruption of flow sensing and tubulogenesis; transepithelial fluid secretion driven by the chloride channel cystic fibrosis transmembrane conductance regulator (CFTR); increased expression of water channels (aquaporin-2, AQP2); and transcriptional regulation of mediators involved in cell proliferation.

transducing calcium-dependent information in vasopressin neurons could be defective in ADPKD. Alternatively, the functional loss of polycystins could affect the level of vasopressin in brain, or interfere with thirst.

VASOPRESSIN AND COPEPTIN IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

The vasotocin–vasopressin and the isotocin–mesotocin–oxytocin lineages evolved from a common ancestral molecule when vertebrates and invertebrates diverged from archemetazoa about 500 million years ago [18,19]. These extraordinarily conserved peptides were recently shown to be crucial to monitor environment and modulate salt chemotaxis in Caenorhabditis elegans [20**]. Vasopressin and oxytocin act on diversified G protein-coupled receptors (GPCRs) that mediate different cellular responses in many tissues (Table 1). Mammals have three vasopressin receptors, V1a and V1b (coupled to a Gaq protein with phospholipase C activation, phosphoinositide hydrolysis and calcium release as second messenger) and V2 (coupled to a Gas protein with cAMP as second messenger). In addition to signaling through activation of heterotrimeric G proteins with a, b and g subunits, GPCRs also signal through G protein-coupled receptor kinase-mediated phosphorylation and b-arrestin binding [21]. Considering the importance of vasopressin throughout evolution, its role in numerous cellular functions (e.g. proliferation and survival; cytoskeletal dynamics, cell adherence and migration; centrosomal separation, bipolar mitotic spindle formation and planar cell polarity) and the number of downstream signaling pathways, its involvement in ADPKD, beyond regulation

Table 1. Vasopressin receptor subtypes and functions

Subtype	Location	Function
V_{1A}	Vascular smooth muscle	Vasoconstriction, myocardial hypertrophy
	Platelets	Platelet aggregation
	Hepatocytes	Glycogenolysis, ureagenesis
	Myometrium	Uterine contraction
	Vasa recta	Decreased blood flow to inner medulla[a]
	Medullary interstitial cells	Stimulation of prostaglandin synthesis[a]
V_{1B}	Anterior pituitary gland	Releases ACTH, prolactin, endorphins
V_2	Collecting duct	Increased water permeability (effect on AQP2)[a]
		Increased sodium reabsorption (effect on ENaC)[a]
		Increased urea permeability (effect on UT-A1)[a]
	Thick ascending limb of Henle	Increased sodium reabsorption[a]
	Vascular endothelium	Releases von Willebrand Factor and Factor VIII
		Vasodilatation

ACTH, adrenocorticotropic hormone; AQP2, aquaporin-2; ENaC, epithelial sodium channel; UT-A1, urea transporter A1.
[a]Contributing to control of water homeostasis.

of water and solute transport, is not surprising. Selected signaling pathways and transcription factors implicated in the pathophysiology of ADPKD, downstream from vasopressin receptors are as follows:

(1) Gas, cAMP, protein kinase A (PKA), exchange protein activated by cAMP, cAMP gated channels
(2) Gaq, phospholipase C, phosphatidylinositide 3-kinase (PI3K), protein kinase B (Akt or PKB), $Ca^{2Þ}$, $Ca^{2Þ}$/calmodulin-dependent protein kinase, calcineurin, nuclear factor of activated T-cells (NFAT)
(3) Gbg canonical and noncanonical signaling
(4) G protein-coupled receptor kinase, b-arrestin, extracellular signal-regulated kinase 1/2 (ERK1/2)
(5) PKA, aquaporin-2, cystic fibrosis transmembrane conductance regulator (CFTR), urea transporter A1
(6) RhoA phosphorylation, Rho kinase inactivation, F-actin depolymerization
(7) Rap1gap, Raf1, mitogen-activated protein kinase (MEK), ERK
(8) PKA, $Ca^{2Þ}$, PI3K, Akt, B-Raf, MEK, ERK
(9) Other mitogen-activated protein kinase (MAPK) family members [c-Jun NH_2-terminal kinase (JNK2), p38a, ERK5]
(10) AMP-activated protein kinase inactivation
(11) MAPK, tuberin, Ras homolog enriched in brain, mammalian target of rapamycin (mTOR)
(12) Glycogen synthase kinase 3b (GSK3b), Wnt, b-catenin
(13) Bad, Boq, other apoptosis related proteins
(14) Transcription factors [cAMP response element-binding protein (CREB), activating protein 1 (AP1), NFAT, signal transducer and activator of transcription 3 (STAT3), Paired box gene 2 (Pax2), etc.]

Vasopressin and oxytocin derive from precursor proteins that consist of a signal peptide, a neuropeptide, a Lys-Arg amino acid cleavage site, and a neurophysin. Preprovasopressin additionally contains a C-terminal glycoprotein (or copeptin) that follows the neurophysin sequence. The neuropeptide, neurophysin and copeptin are separated during the transport of secretory granules and secreted in an equimolar ratio. Although vasopressin is rapidly cleared from plasma, binds to platelets and is unstable ex vivo, copeptin is stable and has been shown to be a reliable surrogate for circulating vasopressin concentration [22]. Cross-sectional analyses of ADPKD patients [23,24] with CKD stage 1–4 showed that serum levels of copeptin are associated with markers of disease severity and with a decrease in GFR. A larger, longitudinal study of 251 ADPKD patients with CKD stage 1–2 [25*] showed a significant association between serum copeptin and changes in kidney volume or decline in GFR after adjusting for sex, age, cardiovascular risk factors, diuretic use, and baseline TKV. Thus, copeptin may help to identify ADPKD patients at risk for rapid disease progression. It should be noted, however, that the physiological role of copeptin remains unknown.

ROLE OF 3′-5′-CYCLIC ADENOSINE MONOPHOSPHATE IN POLYCYSTIC KIDNEY DISEASE

Levels of cAMP are consistently elevated in kidneys of animal models of PKD [26–29,30**]. Proposed mechanisms include: reduction in intracellular calcium due to disruption of the polycystins which in turn activates calcium inhibitable AC6 and inhibits calcium/calmodulin dependent PDE1 (also increasing the levels of guanosine-3′,5′-cyclic monophosphate, cGMP) and cGMP inhibitable PDE3 [27,31]; dysfunction of a ciliary protein complex which normally constrains cAMP signaling via inhibition of AC5/6 activity by polycystin-2 mediated calcium entry and cAMP degradation by PDE4C under the regulation of hepatocyte nuclear factor 1b [32]; depletion of endoplasmic reticulum calcium stores that triggers oligomerization and translocation of stromal interaction molecule 1 to the plasma membrane wherein it recruits and activates AC6 [33*]; other contributory factors such as disruption of polycystin-1 binding to heterotrimeric G proteins, upregulation of V2R, increased levels of vasopressin or accumulation of forskolin, ATP or other adenylyl cyclase agonists in cyst fluid [34–37]. A recent study showing marked inhibition of cystogenesis in a conditional Pkd1 model has confirmed the importance of AC6 in the pathogenesis of ADPKD [38].

The upregulation of cAMP signaling plays a central role in the pathophysiology of ADPKD mainly through activation of PKA and downstream effectors (Fig. 1). PKA activates the CFTR channel and stimulates chloride and fluid secretion [39,40]. Under normal conditions, activation of PKA inhibits mitogen-activated protein kinase (MAPK) signaling and cell proliferation. However, in PKD or in conditions wherein intracellular calcium is reduced, PKA activates MAPK kinase (MEK) in a Src, Ras and B-raf-dependent manner. MEK in turn phosphorylates and activates MAPK, also known as extracellular signal-regulated kinase (ERK) [41,42]. Src and ERK also mediate downstream signaling from b-arrestin and from growth factors and their receptor tyrosine kinases that are upregulated in ADPKD [43]. Therefore, GPCR and receptor tyrosine kinase signaling converge in the activation of c-Src, a non-receptor tyrosine kinase. In the setting of reduced intracellular calcium, PKA also activates CREB signaling and, downstream from ERK and CREB, AP1 that upregulates amphiregulin and other EGF like factors that further promote growth [44]. PKA is also implicated in activation of mTOR (via ERK-mediated phosphorylation of tuberin) [45,46] and Wnt–b-catenin signaling (via phosphorylation of GSK3b and b-catenin) [47,48]. Also, PKA activation interferes with Wnt dependent tubulogenesis [49*], increases ciliary length [50], leads to centrosomal amplification [51], and upregulates STAT3 [52] and possibly Pax2 signaling [53*], all features observed in PKD. Through these multiple pathways, upregulation of cAMP and PKA signaling triggers cell proliferation and apoptosis, enhances fluid secretion, disrupts the control of tubular diameter, and induces cystogenesis.

RATIONALE FOR V2 RECEPTOR ANTAGONISM IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

Given its central role, there is a strong rationale to lower cAMP in cystic tissues. Blocking the effect of vasopressin on V2R is particularly appealing: V2R are almost exclusively located on collecting ducts, connecting tubules, and thick ascending limbs of Henle [54,55], the main sites of cystogenesis, thus minimizing off-target toxicities. Vasopressin is the major GPCR responsible for cAMP generation in isolated collecting ducts [56]. The kidneys are continuously exposed to the tonic action of vasopressin to avoid dehydration. This exposure is further enhanced in PKD, with defective intracellular processes causing cAMP generation and PKA activation (see above).

V2R antagonists (mozavaptan and/or tolvaptan) attenuate the progression of PKD in cpk mice [15] and in rodent models of nephronophthisis (pcy mouse) [27], ARPKD (PCK rat) [27,57] and ADPKD-2 ($Pkd2^{-/WS25}$ mouse) [58]. Mozavaptan is also effective in a conditional Pkd1 knockout when treatment is started early following gene deletion [59]. Suppression of vasopressin by high water intake sufficient to achieve a 3.5-fold increase in urine output attenuates the progression of PKD in the PCK rat [60]. Cyst development is markedly inhibited in PCK rats lacking circulating vasopressin (generated by crosses of PCK and Brattleboro rats), whereas administration of the V2R agonist 1-deamino-8-d-arginine vasopressin fully rescues the cystic phenotype [61]. Low concentrations of tolvaptan also inhibit vasopressin-induced chloride secretion and decrease in-vitro cyst growth of human ADPKD cells [62].

CLINICAL TRIALS OF V2 RECEPTOR ANTAGONISTS IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

Small clinical trials were initially conducted to ascertain the safety and pharmacokinetics of tolvaptan in

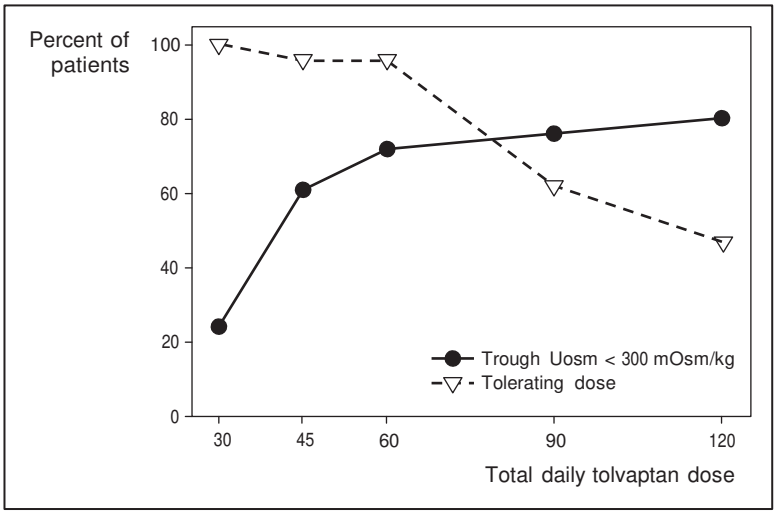

FIGURE 2. Tolerability and efficacy during titration phase with tolvaptan. In the initial 2 months of the TEMPO 2:4 study a split-dose regimen of oral tolvaptan (8 a.m./4 p.m.) was up titrated (15/15, 30/15, 45/15, 60/30, 90/30 mg/d) until tolerability was reached. Tolerability was defined as self-reported tolerance of a specific dose regimen by responding yes to the question: 'could you tolerate taking this dose of tolvaptan for the rest of your life?' Efficacy was defined by the capacity to suppress the action of vasopressin on the kidney reflected by sustained urine hypotonicity (Uosm <300 mOsm/kg). Reproduced with permission from [64].

adult patients with ADPKD [63]. Twice daily administration is necessary to block V2R activation throughout a 24 h period as reflected by urine hypotonicity. A phase 2, open-label, uncontrolled, 3-year clinical trial evaluated the long-term safety and tolerability of tolvaptan in ADPKD [64]. Patients were randomized to one of two doses (45/15 and 60/30 mg) chosen after an analysis of efficacy and self-reported tolerability during titration (Fig. 2). Adverse events were mainly related to aquaresis. Twelve (19%) patients withdrew from the study, in six cases due to adverse events. Changes in TKV (determined by MRI) and eGFR were compared with historical controls from the CRISP and the Modification of Diet in Renal Disease studies. Kidney volume increased 5.8 versus 1.7 %/year and annualized eGFR declined −2.1 versus −0.71 ml/min per 1.73 m^2 per year. Limitations of the study were the small number of patients and the utilization of noncontemporary controls with unmatched ethnicities.

Slight elevations in serum creatinine, rapidly reversible after cessation of drug administration, were observed in phase 2 clinical trials with tolvaptan. The short effects of tolvaptan were investigated in 20 ADPKD patients before and after a split-dose for 1 week [65]. Tolvaptan induced aquaresis was accompanied by significant reduction in iothalamate clearance, increase in serum uric acid due to decreased uric acid clearance, and reduction in serum potassium, without change in renal blood flow. Post-hoc analysis of renal MRIs showed that tolvaptan induced a 3.1% reduction in kidney volume and in the volume of individual cysts.

The results of a phase 3, multicenter, randomized, double-blind, placebo-controlled, parallel-arm trial of tolvaptan in ADPKD (TEMPO, Tolvaptan Efficacy, and Safety in Management of ADPKD and its Outcomes, 3:4), conducted at 129 sites in 15 countries, have been recently published [9,10**]. ADPKD patients (n ¼ 1445) with rapid disease progression reflected by kidney volumes of at least 750 ml at age between 18 and 50 years, but still with preserved renal function (eCrCl >60 ml/min), were randomized 2 to 1 to tolvaptan or placebo. Split 45/15 mg doses of study drug were titrated at weekly intervals to 60/30 and 90/30 mg, if tolerated. The maximally tolerated dose was maintained for 3 years. Serum creatinine and laboratory parameters were measured every 4 months and renal MRIs were obtained yearly. Participants were instructed to drink enough water to prevent thirst. Twenty-three percent of tolvaptan-treated patients withdrew from the trial, 15% due to adverse events including aquaresis-related symptoms in 8%. The corresponding percentages in the placebo group were 14, 5 and 0.4%. Of the patients randomized to tolvaptan who completed the 3 years of treatment, 55% were tolerating the highest dose.

The analysis of the primary endpoint showed that tolvaptan reduced the rate of kidney growth by 50%, from 5.5 to 2.8% per year (Fig. 3). The treatment effect of tolvaptan was greatest from baseline to year one, but it was also significant from year 1 to

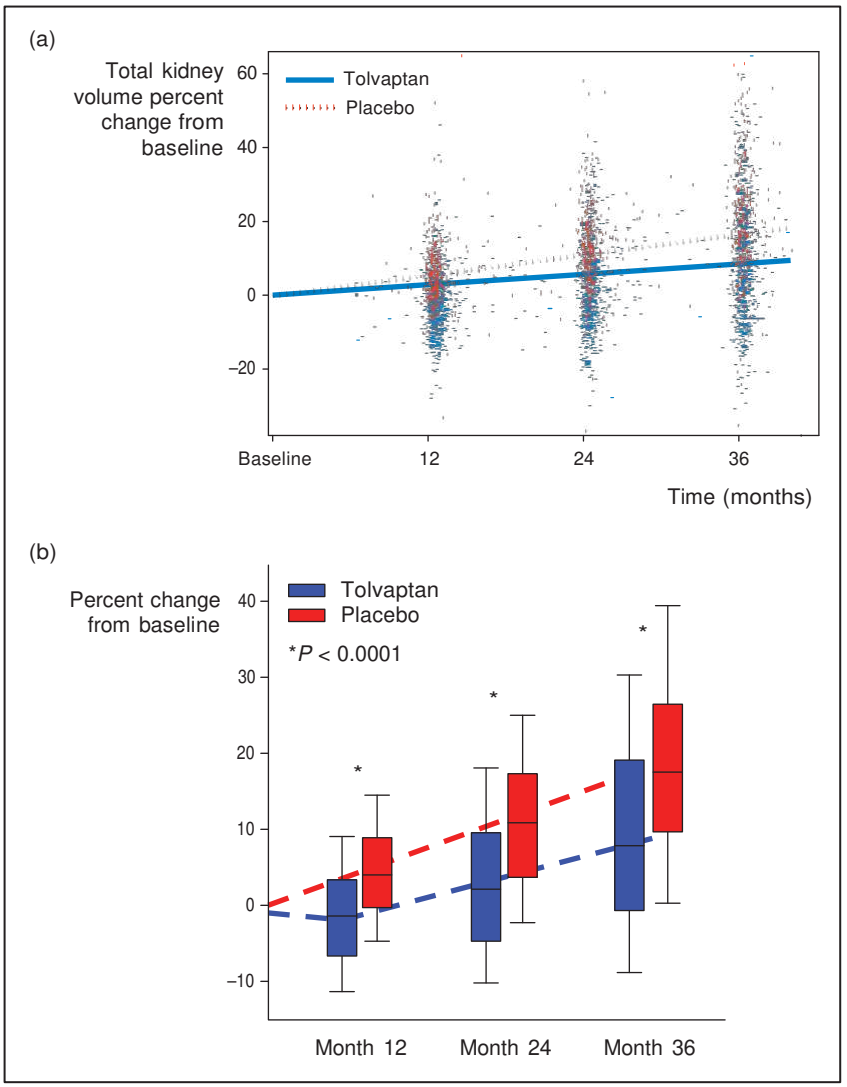

FIGURE 3. Effect of tolvaptan on total kidney volume in autosomal dominant polycystic kidney disease. (a) The slopes of the growth in total kidney volume in the intention-to-treat population during the 3-year treatment period; tolvaptan reduced the rate of kidney growth from 5.5 to 2.8% per year ($P < 0.001$). (b) The treatment effect of tolvaptan was greatest from baseline to year one, but it was also significant from year 1 to 2, and from year 3 to 4, resulting in an increasing separation in kidney volume over time. Reproduced with permission from [10&&].

2, and from year 3 to 4 (Fig. 3). The analysis of the key composite secondary endpoint of time to development or progression of multiple clinical events (worsening kidney function, severe kidney pain, hypertension, and albuminuria) showed fewer clinical events for tolvaptan compared with placebo, with a hazard ratio of 0.87. This positive result was driven by favorable effects on kidney pain and kidney function decline (Fig. 4). The administration of tolvaptan was associated with a 61% lower risk of a 25% reduction in reciprocal serum creatinine and a 36% lower risk of kidney pain. The administration of tolvaptan also reduced the rate of decline of reciprocal serum creatinine, from 3.81 to 2.61 per year (Fig. 4).

The frequencies of adverse events were similar in both groups. Adverse events related to aquaresis were more common with tolvaptan, whereas adverse events related to ADPKD (kidney pain, hematuria, and urinary tract infection) were more common with placebo. Increases in serum sodium and uric acid were more frequent with tolvaptan. Tolvaptan-treated patients had more frequent elevations of liver enzymes, which led to discontinuation of the drug in 1.8%.

At the present time tolvaptan is not approved for the indication of ADPKD and should not be administered to these patients outside of an approved research study. The value of tolvaptan as a long-term treatment in ADPKD will depend

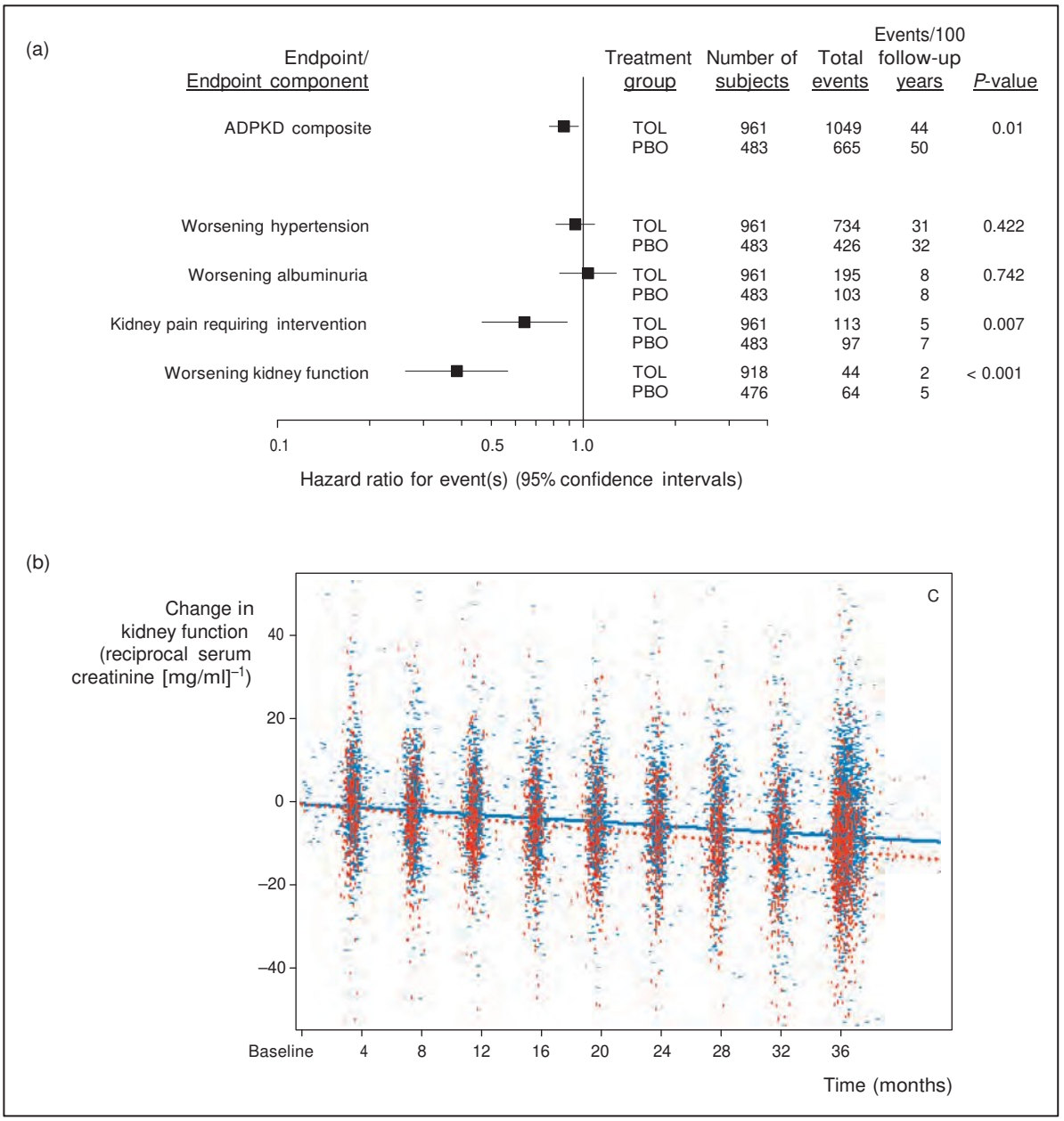

FIGURE 4. Effects of tolvaptan on secondary endpoints and change in kidney function. (a) Hazard ratios for the secondary end point of autosomal dominant polycystic kidney disease (ADPKD)-related events with tolvaptan as compared with placebo for the secondary composite end point and its component events. (b) The slopes of kidney function were estimated with the use of the reciprocal of the serum creatinine level in the intention-to-treat population during the treatment period. The administration of tolvaptan also reduced the on treatment rate of decline of reciprocal serum creatinine, from 3.81 to 2.61 per year ($P < 0.001$). Reproduced with permission from [10••].

on the balance between benefits and risks. Polyuria, thirst and related adverse events may impact the ability of some patients to tolerate effective doses. Patients taking tolvaptan should have easy access to and be able to tolerate water. Levels of plasma sodium and uric acid require monitoring. Liver function should be monitored closely during therapy. Patients in TEMPO 3:4 had relatively preserved renal function. Efficacy in more advanced stages of the disease has not been thoroughly ascertained.

ALTERNATIVE APPROACHES TO TARGET 3′-5′-CYCLIC ADENOSINE MONOPHOSPHATE

A number of GPCRs, in addition to V2R, may affect the generation of cAMP and potentially cystogenesis

in ADPKD. Somatostatin receptors and to a lesser extent secretin, prostaglandin E2 (PGE2), and purinergic receptors have received attention. Somatostatin acts on five GiPCRs (SSTR1–5) present on renal tubular epithelial cells [66]. As somatostatin has a half-life of approximately 3 min, more stable synthetic peptides (octreotide, lanreotide, and pasireotide) have been developed for clinical use. Octreotide and lanreotide bind to SSTR2 and SSTR3, whereas pasireotide has high affinity for SSTR1–3 and SSTR5. In preclinical studies, octreotide and pasireotide halt the expansion of hepatic cysts from PCK rats in vitro and in vivo [67,68]. Similar effects were observed in the kidneys. Three randomized, placebo-controlled studies of octreotide or lanreotide have been completed [69–74]. These drugs induce small, but significant and sustained, reductions in liver volume associated with improved perception of bodily pain and physical activity, and slow kidney growth at least during the first year of treatment. Additional clinical trials for ADPKD and for polycystic liver disease are currently active.

Secretin acting on its Gs-coupled receptor stimulates urine concentration in wildtype and vasopressin-deficient Brattleboro rats at pharmacologic doses [75▪]. However, administration of exogenous secretin to PCK or $Pkd2^{-/WS25}$ mice and genetic elimination of the secretin receptor in $Pkd2^{-/WS25}$ mice had no detectable benefit on the development of polycystic kidney or liver disease [75▪]. Therefore, it seems unlikely that secretin receptor blockers would be valuable to treat ADPKD.

Of the four PGE2 specific E-prostanoid receptors, E-prostanoid 2, and E-prostanoid 4 are coupled to Gas and E-prostanoid 3 to Gai proteins [76]. PGE2 stimulates cell proliferation, fluid secretion, and in-vitro cystogenesis via preferentially expressed E-prostanoid 2 in human ADPKD cells [77], whereas it exerts similar effects via preferentially expressed E-prostanoid 4 in IMCD-3 cells [78]. In a different study, however, PGE2-stimulated proliferation and fluid secretion by ADPKD-1 cultured epithelial cells via activation of E-prostanoid 4 receptors [79]. E-prostanoid 1, which is coupled to Gaq and calcium mobilization, promotes vasopressin synthesis in the hypothalamus [80]. Whether E-prostanoid 2 or E-prostanoid 4 antagonists or E-prostanoid 3 or E-prostanoid 1 agonists affect the development of PKD in vivo has not been investigated.

Purinergic receptors encompass adenosine sensitive P1 and ATP sensitive P2 receptors. Two P1 receptors (A1 and A3) are coupled to Gai proteins and two (A2a and A2b) to Gas proteins. Possibly driven by NF-kB activation, A3 receptor is expressed at high levels in ADPKD compared with normal renal tissues. The A3 agonist 2-chloro-N^6-(3-iodobenzyl)-adenosine-5^0-N-methyluronamide lowers cAMP, ERK, and mTOR activation and cell proliferation in ADPKD derived and in PC1-deficient HEK293 cells [81]. The P2Y receptors are GPCRs, enhance cAMP production through receptor-mediated prostaglandin release, and are upregulated in kidneys of a rat model of ADPKD. Nonspecific P2Y receptor inhibitors (reactive blue 2 and suramin) and the P2Y1-specific antagonist MRS2179 inhibit MDCK cyst growth in collagen matrices [82]. The P2X receptors are ATP-gated, calcium-permeable cation channels. A potential role of P2X7 is suggested by the inhibition of cystogenesis by the antagonist OxATP in pkd2 zebrafish morphants [83].

HIGH WATER INTAKE IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

On the basis of the role of cAMP in cyst progression, the ingestion of supplemental water is increasingly considered as a potential treatment for ADPKD [84]. Provided it can be consistent and sustained, high water intake would suppress endogenous vasopressin, lower stimulation of V2R, and decrease cAMP levels in cyst-lining cells. Low endogenous vasopressin would, thus, reduce nonspecific effects (V1a and V1b-mediated) caused by increased endogenous vasopressin associated with chronic use of selective V2R antagonists [85]. A normal capacity to dilute urine has been observed in ADPKD patients with preserved eGFR, suggesting that rapid inhibition of vasopressin release is preserved [13▪,86,87]. The importance of dietary sodium and protein intake to ensure free-water excretion should be emphasized [84]. The relevance of high water intake in ADPKD has been substantiated by an elegant study showing that high water intake for 10 weeks in the PCK rat reduced vasopressin as well as the renal expression of V2R and the cAMP-dependent activation of the MAPK/ERK kinase (MEK)/ERK pathway through the intermediacy of B-Raf, a kinase that phosphorylates and activates MEK [60]. These changes were reflected by a approximately 30% decrease in kidney/body weight ratio and by improved renal function.

Recommendations for intake of water in ADPKD based on preclinical studies have been proposed [84]. High water intake (approx. 3 l/day), sufficient to achieve a low urinary osmolality (<250 mOsm/kg H_2O), can be proposed in ADPKD patients with an eGFR more than 30 ml/min. Exclusions would include patients on severe protein or sodium restriction; those with volume contraction; those taking diuretics or drugs enhancing the release of AVP; or

those presenting abnormal voiding problems. Monitoring plasma sodium should be advised. The intake should be that of nonmineralized water, with no addition of sugar and no caffeine. Patients should split the intake during the daytime, and void frequently. Urine osmolality remains essential to monitor the action of vasopressin, as urinary cAMP levels showed no predictive value [86]. High water intake should not be advised to patients with more advanced CKD (eGFR <30 ml/min). Limitations of high water intake include risk of hyponatremia and poor compliance, as thirst is not driving the fluid intake like in diabetes insipidus or V2R inhibition.

CONCLUSION

Defective urinary concentration is one the first clinical manifestation of ADPKD. The association of ADPKD with impaired osmoregulation has recently been completed by cellular, animal and clinical studies, indicating that vasopressin and upregulation of cAMP signaling play a central role in cystogenesis. For the first time, a treatment using a V2R antagonist was shown to be able to slow kidney growth, with potential benefits on the functional and symptomatic progression in ADPKD patients. These advances open exciting perspectives, not only for the understanding of cystogenesis and cyst progression in ADPKD, but also for more basic questions related to the components of osmoregulation, the role of polycystins in the brain, the cellular pathways regulated by vasopressin and cAMP, and the pleiotropic action of vasopressin. Burning clinical questions are open: which group of patients, and what stage of disease would benefit most from the V2R antagonist? What are the value and potential role of copeptin as a marker of disease progression? What is the optimal extent of vasopressin inhibition to slow ADPKD – and for how long this should be maintained? The extent and consequences of increased endogenous vasopressin levels in cases of chronic V2R inhibition should be assessed, as well as the potential psychologic and social consequences of polyuria, nycturia, or high water intake. Answering these questions will be critical to tailor interventions capable to prevent decline of renal function and improve clinically significant outcomes in ADPKD.

Acknowledgements

The support of the Fonds Alphonse et Jean Forton, the Fonds de la Recherche Scientifique Médicale the ARC 10/15-029, the National Centre of Competence in Research (NCCR) Kidney.CH (OD) and the National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-44863 and DK-090728, Mayo Translational PKD Center (VET) is gratefully acknowledged.

Conflicts of interest

The authors are members (VET, Chair; OD, Member) of the Steering Committee of the TEMPO 3:4 Study. Aside from that, the authors declare that they have no relevant financial interests.

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